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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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22151 U.S. PTO
60/549289

030204

INVENTOR(S)					
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Robert		Danziger		Chicago, Illinois	
Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Inhibiting PDE4B and PDE4D Activity Reduces Blood Pressure in Salt-Sensitive Hypertension					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number: _____					
OR					
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages 15		<input type="checkbox"/> CD(s), Number _____			
<input type="checkbox"/> Drawing(s) Number of Sheets _____		<input type="checkbox"/> Other (specify) _____			
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.		FILING FEE Amount (\$) \$80.00			
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees.					
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502384					
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____					

[Page 1 of 2]

Respectfully submitted,

SIGNATURE

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TELEPHONE 312-996-7779

Date February 27, 2004

REGISTRATION NO. _____

(if appropriate)

Docket Number: CX049 - B

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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I hereby certify that the foregoing Provisional Patent Application is being deposited with the United States Postal Service, Express Mail Certificate Number EV 222768254 US, postage prepaid, is an envelope addressed to: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 this 27th day of February, 2004.

A handwritten signature in black ink, appearing to read 'G. Comstock', written over a horizontal line.

Gordon L. Comstock
Director, Medicine Intellectual Property

**PROVISIONAL APPLICATION FOR UNITED STATES LETTERS PATENT
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. UIC CX049-B)**

Inventors: Robert S. Danziger
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Assigne: Board of Trustees of the University of Illinois
Chicago, Illinois

Title: Inhibiting Pde4b And Pde4d Activity Reduces Blood Pressure In
Salt-Sensitive Hypertension

**INHIBITING PDE4B AND PDE4D ACTIVITY
REDUCES BLOOD PRESSURE IN SALT-SENSITIVE HYPERTENSION**

BACKGROUND OF THE INVENTION

This provisional application is filed in two (2) sections. The section 1 is a provisional application filed February 20, 2004. Section 2 is a list of additional inhibitors of Pde4b and Pde4d activity.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternative equivalent thereto are within the spirit and scope of the invention as set forth.

SECTION 1

**INHIBITING PDE4B AND PDE4D ACTIVITY
REDUCES BLOOD PRESSURE IN SALT-SENSITIVE HYPERTENSION**

BACKGROUND OF THE INVENTION

There is evidence that increased levels of cyclic adenosine monophosphate (cAMP) both reduces renal vascular resistance and promotes secretion of electrolytes. However, the role that the metabolism of cAMP plays in physiological salt-adaptation and hypertension is not known. We show that PDE4B and PDE4D inhibition reduces blood pressure in a model of salt-sensitive hypertension.

Dibutyryl cAMP increases both renal blood flow and urinary Na⁺ excretion. Relaxant properties of cAMP and beta-adrenergic signaling in arterial beds and in the renal resistance arteries in particular are well established ^{1,2}. Inhibitors of cAMP-dependent protein kinase (Pka) and the Rp diastereomer of adenosine 3',5'-cyclic monophosphorothioate block fluid secretion of both fluids and electrolytes from the inner medullary collecting ducts (IMCD)³. cAMP agonists inhibit renal Na/K ATPase activity via a Pka-phospholipase A2 ^{4,5}. However, the role that renal cAMP plays in physiological salt-adaptation and hypertension is not known. The following work shows that renal cAMP mediates salt-adaptation and plays a mechanistic role in salt-sensitive hypertension.

The components of beta-adrenergic signaling pathway, which is believed to be the primary source of cellular cAMP, are known. Beta-adrenergic agonists stimulate, through G-protein mediated coupling, adenylate cyclase, which converts ADP to cAMP. cAMP in turn stimulates specific protein kinases, i.e., PkAs, that phosphorylate a variety of proteins. Specific PDEs metabolize cAMP to adenosine monophosphate. Thus, PDEs are in central position to regulate cAMP signaling. However, the role that PDEs play in normal physiological processes in general and specifically in salt-adaptation and hypertension are largely known. We answer this in the present work by showing that renal PDEs plays a mechanistic role in blood pressure control.

The cyclic nucleotide PDEs comprise a large number of different isozymes with variable specificities for cyclic guanosine monophosphate (cGMP) and cAMP. The PDE4 family is characterized by a specificity and high affinity for cAMP and consists of four individual isogenes, PDE4A, 4B, 4C, and 4D ^{10,11}. Three of these have been shown to express multiple proteins by virtue of alternate splicing and start sites ¹²⁻¹⁴. PDE4 activity has been reported in kidney, gonads, cardiac muscle, liver, tracheal smooth muscle, various regions of the brain, and in inflammatory cells. Specific PDE4B splice variant have been found to be preferentially expressed in the membrane (PDE4B2) and the cytosol (PDE4B1) of the brain ¹⁵. These two isoforms differ in their N-terminal domains, suggesting that differences in this

domain can determine subcellular localization of the protein ¹⁶. However, the physiological significance of the different isoforms is unknown. In our work, a function of renal PDE4B and/or PDE4D, is demonstrated.

Our work shows that PDE4, specifically PDE4B and PDE4D are therapeutic targets for salt-sensitive hypertension and that inhibiting PDE4 activity reduces blood pressure in salt-sensitive hypertension. This represents an important advance from the work of Tanahashi et al ²⁶, which shows that in the dog kidney, PDE4 participates in the degradation of cAMP and that infusion directly into the renal artery of rolipram, an inhibitor of PDE4, enhances glomerular filtration and urinary Na⁺ excretion, by 1) examining rolipram in salt-adaptation and salt-sensitive hypertension; 2) using an intraperitoneal (leading to systemic) delivery method for rolipram; 3) demonstrating that rolipram is a potent anti-hypertensive agent; and 4) discovering that renal PDE4B and PDE4D are the targets for rolipram in hypertension.

Several therapeutic targets and agents have been identified for hypertension, e.g., angiotensin converting enzyme (ACE) and ACE inhibitors; angiotensin and angiotensin receptor blockers; aldosterone and aldosterone antagonists; and central nervous system and methyl-DOPA. The present work advances the treatment of hypertension by discovering a new therapeutic target for the treatment of hypertension. Agents which act on the target, PDE4B,

may be useful alone and/or provide additional efficacy to existing combination therapy.

The present discovery that PDE4 is a therapeutic target for hypertension leads to new uses for drugs and compounds that inhibit it. Thus, in addition to being of potential utility for disease such as COPD and asthma, they may be useful for arterial hypertension and salt-sensitive hypertension.

DESCRIPTION

Fig. 1 is the continuous telemetric blood pressure monitoring for 6 days in a single Dahl salt-sensitive/Jr rat, a genetic model of renal salt-sensitivity, on 8% NaCl diet with single intraperitoneal injection of rolipram (10 mg/kg) at day 2 (arrow).

Fig. 2 shows the average blood pressures during continuous telemetric monitoring of Dahl SS/Jr rats on 8% NaCl diet before and after a single intraperitoneal injection of rolipram (10mg/kg).

Figures 1 and 2 show that a single injection rolipram causes a very significant and prolonged (> 2 days) drop in blood pressure. Since rolipram inhibits PDE4 and the Dahl SS/Jr possesses renal salt-sensitive hypertension, this links PDE4 activity to salt-sensitive hypertension.

Fig. 3 is the renal PDE4B abundance in Dahl SR and SS rats on 8% and 0.3% NaCl diets for 10 days. Representative Western analysis using a PDE4B anti rabbit polyclonal antibody to PDE4 which detects PDE4B and splice variants showing greatest abundance of PDE4B1 splice variant.

Fig. 4 is a bar graph showing abundance of PDE4B1 determined in Dahl SS/Jr and salt-resistant (SR) rat kidneys by Western analysis using a polyclonal anti-rabbit antibody which reacts with various PDE4B splice variants ($n = 3$; $P < 0.05$).

Figures 3 and 4 show that PDE4B, a primary target of rolipram in general, is in the kidney of the Dahl SS/Jr rat, demonstrating that this isoform is a target of rolipram in the kidney of the Dahl SS/Jr rat. Figure 4 shows specifically that PDE4B1 abundance is influenced by dietary salt content, suggesting that it plays a mechanistic role in salt-adaptation and is a primary target for rolipram in reducing blood pressure.

Fig. 5 is a representative Western blot using PDE4D anti-rabbit antibody which detects PDE4D splice variants showing the presence of PDE4D5 in the kidney. Abundance is less in Dahl SR kidney from rat on 8% versus 0.3% NaCl diets. This demonstrates that PDE4D5 is present in the rat kidney and appears to play a mechanistic role in salt adaptation. Thus PDE4D5 is also a renal target of rolipram.

Figure 1

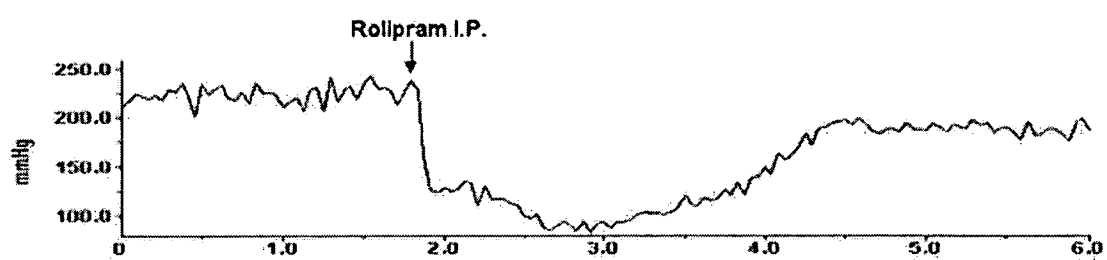


Figure 2

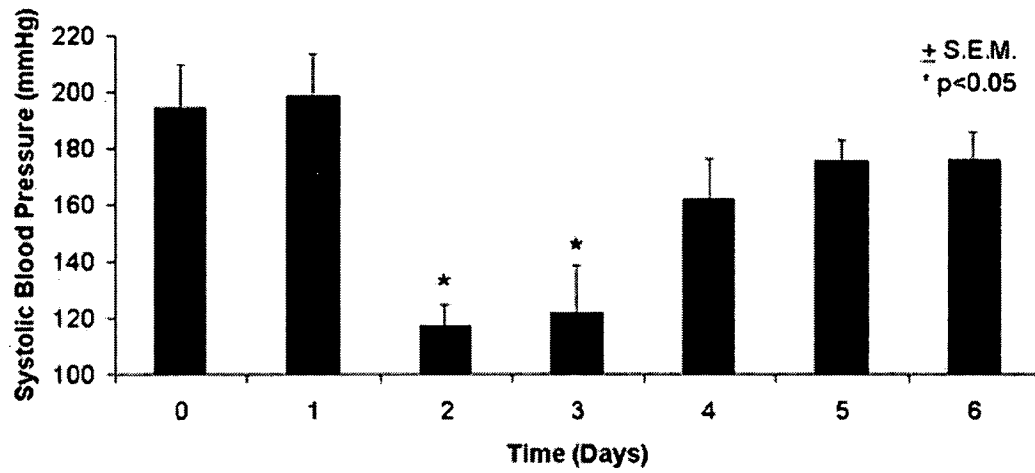


Figure 3

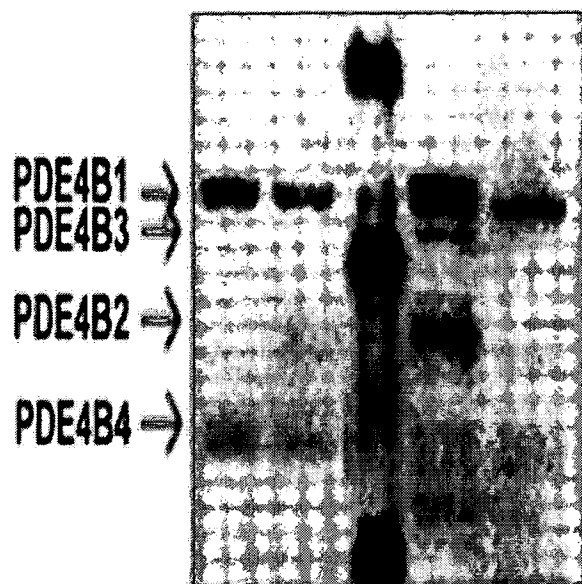


Figure 4

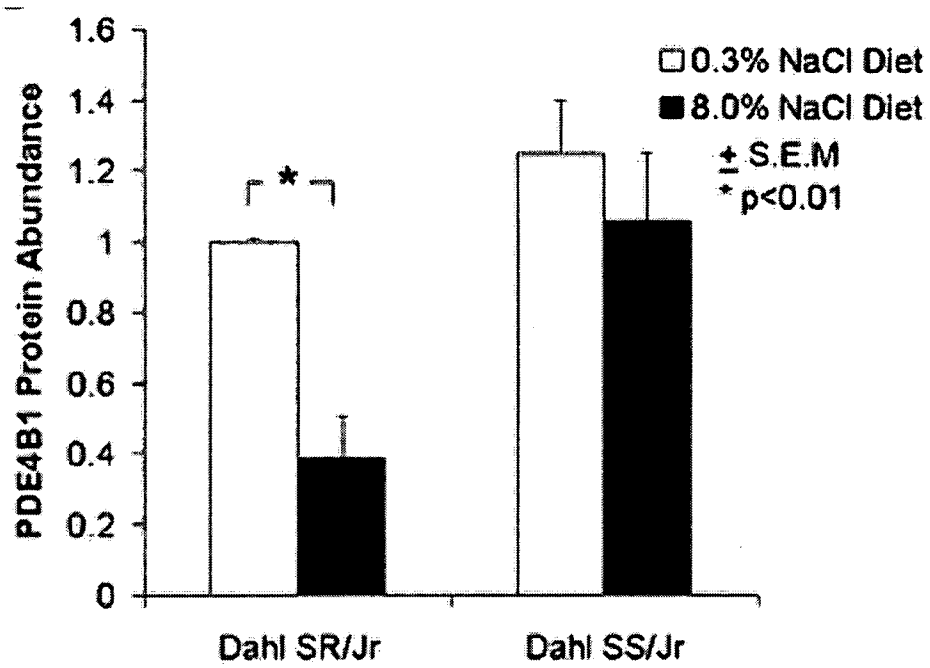


Figure 5

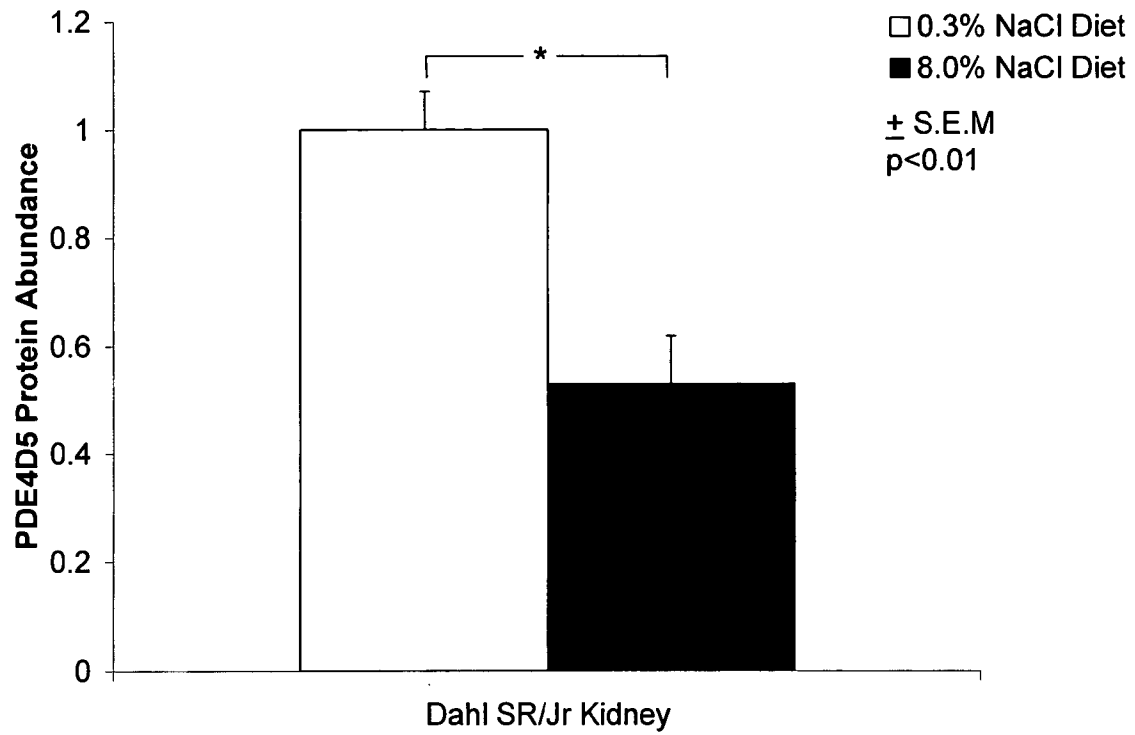


Figure 5

SECTION 2

Additional inhibitors of Pde4b And Pde4d activity that reduces blood pressure in hypertension.

1. N-substituted cis-tetra- and cis-hexahydrophthalazinones
2. Substituted aminopyridines
3. L-791,943
4. TVX2706
5. RP73401
6. RS25344